UNITED STATES OF AMERICA

TO ALL WHOM IT MAY CONCERN:

BE IT KNOWN THAT WE, ARTHUR EDWARD DIXON, 601 Stonebury Crescent, Waterloo, Ontario, Canada, N2K 3R2, Canadian Citizen, SAVVAS (nmi) DAMASKINOS, 8 Lennox Crescent, Waterloo, Ontario, Canada, N2N 2H3, Canadian Citizen, and Brian C. Wilson, 85 Indian Grove Road, Toronto, Ontario, Canada, M6R 2Y6, Canadian Citizen, have invented certain new and useful improvements in

IMAGING SYSTEM HAVING A FINE FOCUS, of which the following is a specification:-

## BACKGROUND OF THE INVENTION

### FIELD OF THE INVENTION

This invention relates to the fields of confocal and non-confocal imaging of microscopic and macroscopic samples with particular emphasis on scanning beam fluorescence and photoluminescence imaging systems, including multi-photon fluorescence imaging and spectrally-resolved fluorescence imaging. Applications include imaging tissue specimens, genetic microarrays, protein arrays, tissue arrays, cells and cell populations, biochips, arrays of biomolecules, and many others. Other applications of this optical system include photodynamic therapy, image-guided microsurgery, and many others.

### DESCRIPTION OF THE PRIOR ART

**Figure 1** shows one embodiment of a prior art confocal scanning laser macroscope, as described in US Patent **#5,760,951**. In this embodiment, the incoming laser beam **101** from laser **100** passes through a spatial filter and beam expander (comprised of lens **102**, pinhole **104** and lens **106**), and is expanded to match the diameter of the entrance pupil **112** of laser scan lens **118** (note – entrance pupil **112** as indicated on the figure simply indicates the position of the entrance pupil. A real stop is not usually placed at this position). Scanning mirrors **110** and **116** deflect the beam in a raster scan, and rotate about axes that are perpendicular to each other. These mirrors are placed close together, on either side of the entrance pupil of the laser scan lens. Laser scan lens **118** focuses the beam to a spot on the sample **120**, and reflected light is collected by laser scan lens **118**, descanned by scanning mirrors **116** and **110**, and partially reflected by beamsplitter **108** into a confocal detection arm comprised of lens **128** and pinhole **130**. A detector **132** is located behind the pinhole **130**.

Light reflected back from the focused spot on the sample passes through pinhole **130** and is detected, but light from any other point in the sample runs into the edges of the pinhole and is not detected. The scan mirrors are computer-controlled to raster the focused spot across the sample. A computer, represented by computer screen **134**, is connected to the detector **132** to store and display a signal from detector **132**. The computer provides means for displaying the signal from the detector. This confocal macroscope has properties similar to those of a confocal scanning laser microscope, except that the field of view of the microscope is much smaller.

Several other embodiments of the macroscope are presently in use. These include instruments for fluorescence and photoluminescence (including spectrally-resolved) imaging (several other contrast mechanisms are also possible), instruments in which a stage scan in one direction is combined with a beam scan in the perpendicular direction, non-confocal versions, and other embodiments. The combination of a scanning laser macroscope with a scanning laser microscope to provide an imaging system with a wide field of view and the high resolution capability of a microscope was described in US Patent # 5,532,873.

The prior art macroscopes described herein and in the literature have some limitations. When focusing the instrument on a specimen, either to achieve best focus or for confocal slicing, focus position is achieved by changing the distance between the specimen and the laser scan lens. This is usually accomplished by raising or lowering the specimen on an adjustable or motorized specimen stage, or by raising or lowering the laser scan lens (or the macroscope itself) relative to the specimen. Some specimens are difficult to move, or too large to be

placed on a specimen stage (one example is the human body, when the instrument is used for in-vivo imaging). This makes fine focus motion difficult to accomplish, and in the case of a macroscope using a liquid-immersion laser scan lens, changes the distance between the scan lens and the specimen, making it difficult to maintain a uniform layer of immersion fluid between the scan lens and specimen.

## SUMMARY OF THE INVENTION

It is an object of this invention to provide a confocal or non-confocal imaging system for macroscopic samples in which the coarse focus is achieved by moving the sample and the laser scan lens relative to one another, and fine focus (or confocal slicing) is achieved by adjusting the position of a lens in the intermediate optics (note could use reflecting intermediate optics as well). This will be particularly important for in-vivo imaging including using a macroscope containing a liquid-immersion laser scan lens for in-vivo imaging.

It is an object of this invention to provide a confocal or non-confocal imaging system for macroscopic samples in which the coarse focus is achieved by moving the sample and the laser scan lens relative to one another, and dynamic fine focus is achieved by adjusting the position of a lens in the intermediate optics while the scan is underway.

It is a further object of this invention to provide an instrument that controls the position of the moving focused laser spot (volume) to move it along a previously defined path inside a sample volume defined by the area of the field of view of the laser scan lens in two dimensions and the range of axial fine focus in the third (perpendicular) direction achieved by moving a lens in the intermediate optics.

It is a further object of this invention to provide an apparatus and method for performing image-guided microsurgery using a laser for cutting (excising, ablating or resecting) tissue.

It is a further object of this invention to provide an apparatus and method for performing image-guided microsurgery using a short pulse laser to generate multi-photon absorption for cutting (excising, ablating or resecting) tissue.

It is a further object of this invention to provide an apparatus and method for image-guided photodynamic therapy.

It is a further object of this invention to provide a scanning beam optical instrument for multi-photon fluorescence imaging.

It is a further object of this invention to provide an apparatus and method for exciting a small volume inside a semiconductor specimen (including a semiconductor device) for optical beam induced current generation, or device repair or testing.

It is a further object of this invention to provide an apparatus and method for exciting a small volume inside a semiconductor specimen (including device) for device repair or testing or optical beam induced current generation using a short pulse laser that results in multi-photon (or two photon) absorption at the focus volume inside the semiconductor.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure **1** is a schematic view of a prior art confocal scanning-beam optical macroscope.

Figure **2a** is a schematic view of a confocal macroscope having a fine focus control;

Figure **2b** is a schematic view of a confocal macroscope having a liquid immersion lens with a fine focus control;

Figure **3a** is a schematic view of a multi-photon macroscope having a fine focus control;

Figure **3b** is a schematic view of a multi-photon macroscope with a transmission detector and a fine focus control;

Figure **4a** is a schematic view of a confocal scanning laser macroscope having a fine focus control;

Figure **4b** is a schematic view of a multi-photon scanning laser having a fine focus control;

Figure **5a** is a schematic view of a confocal scanning laser macroscope having a liquid immersion scan lens and a flexible dam with a fine focus control; and

Figure **5b** is a schematic view of a multi-photon scanning laser macroscope having a liquid immersion scan lens and a flexible dam with a fine focus control.

## DESCRIPTION OF A PREFERRED EMBODIMENT

When the word "object" is used in the present application, it includes any subject that is used with an optical imaging system or with a liquid immersion scan lens including, without limiting the generality of the foregoing, a sample, specimen, body or subject including living organisms or parts of a body or subject. The liquid imaging system of the present invention can be used for in-vivo applications.

The present invention is a high-resolution confocal, non-confocal or multi-photon scanning laser macroscope in which coarse focus is achieved by moving the specimen relative to the macroscope scan lens, and fine focus is achieved by moving a lens in the intermediate optics. In a scanning laser macroscope, focus is usually achieved by moving the specimen relative to the scan lens, or moving

the scan lens (or the entire macroscope optical subassembly) relative to the specimen. Some specimens are difficult to move, or too large to be placed on a specimen stage (one example is the human body, when the instrument is used for in-vivo imaging). This makes fine focus motion difficult to accomplish, and in the case of a macroscope using a liquid-immersion laser scan lens, changes the distance between the scan lens and the specimen, making it difficult to maintain a uniform layer of immersion fluid between the scan lens and specimen. In addition, it is easier to obtain rapid focus changes by moving a small lens than by moving the larger scan lens or the specimen stage, and fine focus automation can be obtained by controlling the position of the intermediate focusing lens using a motorized stage. With such motion control, dynamic focus can be achieved for imaging, or the focused laser spot can be moved along any line inside the three-dimensional volume defined by the scan area and the axial fine focus distance. Confocal slicing can also be accomplished by moving the focusing lens on a computer-controlled stage.

The optical diagram of a scanning beam-scanning stage confocal scanning laser macroscope using a movable lens in the intermediate optics for focusing is shown in Figure **2a**. Light beam **101** from laser **100** (or other light source) is expanded by a first beam expander comprised of lenses **201** and **202**, passes through beamsplitter **108** and enters a second beam expander comprised of lenses **204** and **205** (lenses $\mathbf{L_1}$ and $\mathbf{L_2}$, with focal lengths $\mathbf{f_1}$ and $\mathbf{f_2}$). The combination of first and second beam expanders expand the light beam to fill the entrance pupil **112** of the laser scan lens **118**; when the light beam from laser **100** is large enough (more than about a few millimeters in diameter), the first beam expander is not required. Note

that a real stop is not required at the entrance pupil position – **112** simply indicates the size and position of the external entrance pupil of scan lens **118**. The incoming beam is directed toward scan lens **118** by scanning mirror **116**. Scan lens **118** focuses the incoming beam onto specimen **206**, shown mounted on a microscope slide **207**. Microscope slide **207** is mounted on scanning stage **208**. In this scanning beam-scanning stage configuration, a raster scan of the focus spot **213** across the surface of specimen **206** is achieved by scanning the beam in the x direction using scanning mirror **116** and moving the specimen slowly in the y direction using the scanning stage **208**. Other scan mechanisms are possible, including the scanning beam arrangement shown in Figure **1** and other scan mechanisms. Light emitted from, or reflected by, specimen **206** at the focal point **213** is collected by scan lens **118**, descanned by scanning mirror **116**, passes back through lenses **205** and **204**, and is reflected by beamsplitter **108** into a detection arm comprised of filter **203**, detector lens **128** and pinhole **130**. A detector **132** is located behind the pinhole **130**. For fluorescence imaging, a beamsplitter **108** is usually a dichroic beamsplitter, and filter **203** is a laser rejection filter. Beamsplitter and filter combinations depend on the application. In some applications (e.g. reflected light), no filter **203** is required.

The macroscope shown in Figure **2a** has a scanning-beam/scanning-stage configuration. Beam scanner **116** moves the focus spot in the x-direction, while scanning stage **208** moves the specimen in the y-direction.

Lens **204** has been mounted so that it can be moved in the axial direction. When moved to the right (in the figure) from it's nominal position, the expanded beam on the right of lens **205** will be diverging,

causing the scan lens **118** to focus below its nominal focus position. When lens **204** is moved to the left, the expanded beam on the right of lens **205** will be converging, causing the scan lens to focus above its nominal focus position. Coarse focus of the instrument is achieved by changing the distance between the scan lens **118** and the sample **206**. Fine focus of the instrument is achieved by moving lens **204** relative to the scan lens **118**. Focus could also be changed by moving lens **205**; however it is preferable to move lens **204** because it is smaller. Because lens **204** is small, it can be controlled to move rapidly, and this can be used for dynamic focusing while the scan proceeds. This is particularly important when large samples are used (one example is the imaging of cracks and corrosion on metal parts), or for in-vivo imaging where it is difficult to quickly change focus by moving the patient or the scan lens. In-vivo imaging will most likely be performed using a scanning-beam/scanning-beam instrument.

The macroscope shown in Figure **2a** is well suited for imaging genetic microarrays and for tissue and cell imaging, including tissue and cell arrays. When very high resolution is required, the depth of focus of the instrument is small, and dynamic focus can be achieved by moving lens **204** rapidly on a motorized stage under computer control. For example, if a genetic microarray on a glass microscope slide must be imaged at high resolution, without dynamic focus correction the macroscope tends to go out of focus as the scan moves across the width of the microscope slide. Dynamic focus can be achieved by controlling the position of lens **204** as the scan proceeds, and since lens **204** has a small mass, it is much easier to achieve rapid motion of lens **204** than to rapidly move scan lens **118** or microscope slide **207**.

Figure **2b** illustrates a scanning laser macroscope that is similar to that shown in Figure **2a**, except that the laser scan lens **118** has been replaced by a liquid-immersion laser scan lens **212**. In this figure the specimen **206** is mounted in mounting medium **209** under cover glass **210** and the space between the cover glass and the bottom element of scan lens **212** is filled with immersion fluid **211**. The immersion fluid is chosen to have an index of refraction that matches (or nearly matches) the index of refraction of the mounting medium, cover glass and the bottom lens element in the scan lens. Fine focus using a focusing lens in the intermediate optics is particularly important in this case, where a thin film of immersion fluid must be maintained between the scan lens and the cover glass. This is much more difficult to accomplish if the distance between the laser scan lens and specimen changes during focusing.

Figure **3a** illustrates a two-photon (or multiphoton) macroscope. Light beam **315** from Short Pulse Laser **300** (a picosecond or femtosecond or other short pulse laser) is expanded by a beam expander comprised of lens **201** and lens **202**, passes through a beamsplitter **108** (a Dichroic beamsplitter is often used), is expanded by a beam expanding telescope comprised of lenses **204** and **205** to fill the entrance pupil **112** of scan lens **118**, is scanned by scanning mirror **116**, and focused by laser scan lens **118** to a focal spot on specimen **206**. Two-photon (or multiphoton) fluorescence from the specimen (not shown) at the focal spot is collected by scan lens **118**, descanned by scanning mirror **116**, passes back through the telescope and is reflected by beamsplitter **108** into a detection arm comprising laser line rejection filter **310** and condenser lens **301**. A detector **302** is located behind the condenser lens **301** (any filter can be used in place of the

filter **310** as long as it rejects the laser wavelength and passes the fluorescence wavelengths). Note that no confocal pinhole is required since two-photon (or multi-photon) fluorescence is excited only near the focus point of the short pulse laser. If the active area of detector **302** is large enough to intercept all of the light in the returning beam, no condenser lens is required. Lens **204** has been mounted so that it can be moved in the axial direction relative to the scan lens **118**. When moved to the right (in the figure) from it's nominal position, the expanded beam on the right of lens **205** will be diverging, causing the scan lens **118** to focus below its nominal focus position. When lens **204** is moved to the left, the expanded beam on the right of lens **205** will be converging, causing the scan lens to focus above its nominal focus position. Coarse focus of the instrument is achieved by changing the distance between the scan lens **118** and the sample **206**. Fine focus of the instrument is achieved by moving lens **204** relative to the scan lens **118**. Focus could also be changed by moving lens **205**; however it is preferable to move lens **204** because it is smaller. Because lens **204** is small, it can be controlled to move rapidly, and this can be used for dynamic focusing while the scan proceeds. This is particularly important when large samples are used (one example is the imaging of cracks and corrosion on metal parts), or for in-vivo imaging where it is difficult to quickly change focus by moving the patient or the scan lens. In-vivo imaging will most likely be performed using a scanning-beam/scanning-beam instrument.

The scan lens **118** shown in Figure **3a** uses no immersion fluid; however a water-immersion (or other immersion fluid) scan lens can also be used, and the increased NA of the immersion lens will increase

the intensity of the light at the focus, thus improving two-photon (or multiphoton) absorption and fluorescence detection.

Figure **3b** illustrates a two-photon (or multiphoton) macroscope with a transmission detector for transmitted light or multi-photon fluorescence, with fine focus adjustment by moving a lens in the intermediate optics. In this embodiment light transmitted through specimen **206** (or multi-photon fluorescence emitted by specimen **206**) is detected in a detection arm below the specimen. Condenser lens **322** collects light from the focal spot in the specimen, and directs it towards detector **324**. If condenser lens **322** is placed a distance equal to its focal length below the focal plane of the macroscope, and a distance equal to its focal length in front of detector **324**, then the cone of light originating at the focal spot will be a parallel beam directed towards the center of detector **324**, reducing the motion of the incoming light across the surface of the detector. Condenser lens **322** works well if it has a short focal length and large diameter. It has been found that Fresnel lenses work well in this application. For detecting multi-photon fluorescence, a laser line rejection filter **320** is placed between the specimen and the detector. In some applications, detector **324** replaces detector **302** entirely. In others (for example, when the fluorescence wavelengths will not penetrate through a thick specimen) detector **302** will be required. Note that this transmission arm arrangement, comprised of laser line rejection filter 320 (or other filter, depending on the application), condenser lens 322 and detector 324 can also be used for detecting non-confocal transmission or fluorescence with the confocal macroscopes described earlier, and in Figures **4a** and **4b** below.

Figure **4a** illustrates a confocal scanning laser macroscope that is similar to that shown in Figure **2a**, except the scanning-beam/scanning-stage configuration of Figure **2a** has been replaced by a general purpose scanner **400**. This is meant to illustrate that many kinds of scan combinations are possible – scanning-beam/scanning-beam as shown in Figure **1**, scanning-beam/scanning-stage as in Figure **2a**, or any other scanner that results in a raster scan of the focused laser beam across the specimen. The incoming beam can be focused at any point inside the "sampling volume" **402** of volume $\mathbf{D^2t}$ by moving lens **204** (or lens **205**) away from its nominal position a distance $\mathbf{f_1+f_2}$ from lens **205,** without moving either laser scan lens **118** or the specimen relative to one another. This enables the macroscope to form an image of any sample plane inside this sampling volume, and by controlling the scan using scanner **400** and the focus position using lens **204**, the focused spot can be moved to follow any path inside that volume. This embodiment can be used for several applications, including but not limited to image-guided surgery, image-guided microsurgery, image-guided photo dynamic therapy, photoluminescence testing of semiconductor materials and devices, optical-beam-induced-current testing of devices, and two-photon absorption (to create electron-hole pairs or to create defects or repair defects below the surface of the sample) in semiconductor materials and devices or for irradiating a specific area for photo dynamic therapy. The scan lens **118** shown in Figure **4a** uses no immersion fluid; however a water-immersion (or other immersion fluid) scan lens can also be used.

Figure **4b** illustrates a multi-photon scanning laser macroscope that is similar to that shown in Figure **3a**, except the scanning-beam/scanning-stage configuration of Figure **3a** has been replaced by a

general purpose scanner **400**. This is meant to illustrate that many kinds of scan combinations are possible – scanning-beam/scanning-beam as shown in Figure **1**, scanning-beam/scanning-stage as in Figure **2a**, or any other scanner that results in a raster scan of the focused laser beam across the specimen. The incoming beam can be focused at any point inside the "sampling volume" **402** of volume $\mathbf{D^2t}$ by moving lens **204** (or lens **205**) away from its nominal position a distance $\mathbf{f_1+f_2}$ from lens **205,** without moving either laser scan lens **118** or the specimen relative to one another. This enables the macroscope to form an image of any sample plane inside this sampling volume, and by controlling the scan using scanner **400** and the focus position using lens **204**, the focused spot can be moved to follow any path inside that volume. This embodiment can be used for several applications, including but not limited to, image-guided microsurgery, image-guided photo dynamic therapy, photoluminescence testing of semiconductor materials and devices, optical-beam-induced-current testing of devices, and two-photon absorption (to create electron-hole pairs or to repair or create defects below the surface of the sample) in semiconductor materials and devices, or for irradiating a specific area for photo dynamic therapy. The scan lens **118** shown in Figure **4b** uses no immersion fluid; however a water-immersion (or other immersion fluid) scan lens can also be used, and the increased NA of the immersion lens will increase the intensity of the light at the focus, thus improving two-photon (or multiphoton) absorption and two-photon (or multiphoton) fluorescence detection. One particularly useful embodiment for use in surgical applications, or for in-vivo imaging, is a macroscope with this design in which the scan lens **118** is designed to work with water as an

immersion fluid, and the volume $\mathbf{D^2t}$ includes the tissue volume to be imaged and /or cut.

Figure **5a** shows a confocal macroscope that is optimized for in-vivo applications. This macroscope is similar to that shown in Figure **4a** except scan lens **118** has been replaced by immersion scan lens **504**, and immersion fluid **501** (usually water) of index of refraction $\mathbf{n_f}$ is contained inside a flexible dam **501** (a soft rubber or plastic O-ring can be used). The flexible dam forms a barrier to contain the immersion fluid between the bottom of lens **504** and the surface of specimen **503**. Although the focus position is shown at the surface of specimen **503**, the focus position of this macroscope can be adjusted by moving lens **204** such that any focus position inside the imaging volume $\mathbf{D^2t}$ can be reached, including areas inside the specimen **503** that are within the penetration depth of the laser beam. The use of an immersion scan lens results in higher resolution imaging (because of the higher NA of the immersion lens) and higher sensitivity for fluorescence imaging. For in-vivo applications, the instrument is used in reflectance and/or fluorescence mode, or a combination of the two.

Figure **5b** shows a multi-photon macroscope that is optimized for in-vivo applications. This macroscope is similar to that shown in Figure **4b** except scan lens **118** has been replaced by immersion scan lens **504**, and immersion fluid **501** (usually water) of index of refraction $\mathbf{n_f}$ is contained inside a flexible dam **501** (a soft rubber or plastic O-ring can be used). The flexible dam forms a barrier to contain the immersion fluid between the bottom of lens **504** and the surface of specimen **503**. Although the focus position is shown at the surface of specimen **503**, the focus position of this macroscope can be

adjusted by moving lens **204** such that any focus position inside the imaging volume $\mathbf{D^2t}$ can be reached, including areas inside the specimen **503** that are within the penetration depth of the laser beam. Because of its higher NA (for the same field of view), an immersion scan lens increases the intensity of the light at the focus, thus improving two-photon (or multiphoton) absorption and fluorescence detection. This embodiment is particularly useful for several applications, including, but not limited to, in-vivo imaging, image-guided surgery, image-guided microsurgery, image-guided photo dynamic therapy, image-guided surgery and in-vivo multi-photon fluorescence imaging.

When used for fluorescence imaging of tissue, the macroscopes described herein can be used for tissue autofluorescence or with fluorescence agents, including but not limited to fluorescent dyes either alone or linked to a targeting/delivery vehicle or quantum dots (fluorescent nanoparticles).

The macroscopes described herein can be used for fluorescence excitation and emission, and reflection in the ultraviolet, visible and near-infrared wavelength ranges.

The macroscopes described herein can be used for multi-spectral or hyperspectral imaging, in either reflectance or fluorescence mode, by replacing the detector by a spectrally-resolved detector. Multi-spectral or hyperspectral measurements can be made at any point by stopping the scan at that point. Several implementations of spectrally-resolved detectors in a scanning laser microscope are shown in US Patent # 5,192,980, and these implementations will also work in the macroscopes described herein. In reflectance, spectral analysis can extract information on morphological features of cells and tissues. In

fluorescence, spectral analysis will enable the extraction of information on the structural and/or biochemical nature of the tissue. Correction for autofluorescence background can be made in hardware or software.

Both the fluorescence spectra and the lifetimes of fluorophores are sensitive to their local environment, and thus changes in emission spectrum or lifetime as a function of position provide contrast mechanisms that can be used to differentiate between normal, pre-cancerous and cancerous tissue. The macroscopes described herein can be modified for fluorescence lifetime imaging by modulating the light source at a high frequency and using a lock-in amplifier to detect the phase shift and amplitude of the fluorescence emission signal compared to the excitation light.

Both confocal and multi-photon macroscopes are useful for photodynamic therapy, both for delivery of the light beam to the area of therapy and image guidance for that delivery, and for monitoring treatment after therapy. In the cases of image guidance and treatment monitoring, the light-based treatment may or may not be delivered through the macroscope optical system.

The macroscopes described herein will be useful for photoluminescence imaging and optical beam induced current imaging or testing of semiconductor materials and devices. In particular, the multi-photon macroscope, using an infrared laser with photon energy smaller than the semiconductor bandgap energy, can penetrate deeply into a semiconductor and either create a small volume of electron-hole pairs inside the sample, which can be used to test complicated three-dimensional circuits by injecting charge near a device junction, or,

when more intense beams are used, can effect repairs or cause disconnects in the circuitry.

All of the embodiments shown in the figures are based on an infinity-corrected optical design, however non-infinity corrected versions are also possible. Non-telecentric scan lenses can also be used. The light source shown is a laser however other light sources can also be used, including arc lamps and light-emitting diodes. A white light source will be useful in some applications, including brightfield imaging of tissue specimens. Reflecting optics can also be used.

The term scan lens, as used in this document, describes a lens that is normally used for focusing a parallel beam of light to a small spot that scans across the focal plane. The incoming parallel beam is usually directed by a scanner placed at the position of the entrance pupil of the scan lens. Such a lens has a combination of wide angular field, a flat image plane, and an external entrance pupil (at which position a scanning mirror or other scanner is often placed). Although many laser scan lenses are monochromatic, color-corrected scan lenses are most useful in the applications described herein. Many scan lenses include f*theta correction and many are telecentric.

Several embodiments of a novel high-resolution scanning optical macroscope for imaging microscopic and macroscopic specimens have been disclosed. In one embodiment, lthe imaging system has a laser light source that is adjustable and controllable to enable said imaging system to perform at least one of image guided microsurgery, image guided surgery, microsurgery, image guided photo-dynamic therapy, multi-photon fluorescence imaging or to excite a small volume inside a semi-conductor.

Having described preferred embodiments of a new scanning optical macroscope for imaging microscopic and macroscopic specimens, constructed in accordance with the present invention, it is believed that other modifications, variations, and changes will be suggested to those skilled in the art in view of the teachings set forth herein. It is therefore to be understood that all such variations, modifications, and changes are believed to fall within the scope of the present invention as defined by the appended claims.